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Addendum

Cloning and Subcellular Location of an Arabidopsis Receptor-Like Protein That Shares Common Features with Protein-Sorting Receptors of Eukaryotic Cells

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The genomic approach used to clone the receptor-like protein *AtELP* was more complex than indicated in Ahmed et al. (1997). After it was reported by N. Paris at the 1995 Keystone meeting that several homologs of BP-80, a potential vacuolar protein-sorting receptor isolated from pea (Kirsch et al., 1994, 1996), were present in the Arabidopsis, maize, and rice databases, we attempted to identify ESTs for plant protein-sorting receptors. To achieve this, we performed computer searches with various consensus sequences (Tyr motif and various Cys-rich motifs) of receptors involved in endocytosis and protein sorting.

Sequence alignment of the Cys-rich B.2 motif of the animal endocytic receptor low-density lipoprotein receptor superfamily contains a consensus sequence with the longest uninterrupted stretch of five amino acids (NGGCS). Many variations of this consensus sequence occur in various proteins and we searched the Arabidopsis EST database at monthly intervals with a number of variations of this peptide sequence. Specifically, a search of the Arabidopsis EST database with the NNGGC sequence using the Motif Explorer program identified two ESTs (nos. T22799 and T43896). BLAST analysis of T22799 identified another Arabidopsis EST (no. R29853). A search of the GenBank at the National Center for Biotechnology Information with the sequence from R29853 identified two new Arabidopsis ESTs (nos. R90202 and T42090) and three rice ESTs (nos. D40971, D41226, and D40769). All of these ESTs were partial clones and were within the same region of a protein. We obtained all four Arabidopsis ESTs from the Michigan State University, Department of Energy Plant Research Laboratory Genome Sequencing Project in September, 1995 (Newman et al., 1994), and upon restriction digest analysis, we decided to concentrate our efforts on nos. R90202 and R29853.

To obtain a full-length version of R90202, we used an antisense primer corresponding to the 5′ end of the R90202 clone, the T7 primer, and the PRL2 cDNA library (Newman et al., 1994) as a template. A PCR product was isolated that had a partial 3′ end sequence identical to the 5′ end of R90202. Furthermore, the partial 5′ end sequence of this PCR product was used to blast the Arabidopsis database. The 5′ end sequence identified two Arabidopsis EST clones (nos. R30384 and Z38123). We obtained R30384 from Michigan State University and found that it contained the largest insert of all of the five Arabidopsis EST clones (nos. T22799, R29853, R90202, T42090, and R30384). The clones nos. R30384 and R90202 were sent for complete sequencing. Analysis of the sequence indicated that the two contiguous cDNAs shared a 130-bp overlapping region, and when fused, indicated the presence of an open reading frame of 623 amino acids, the exact size of BP-80, as reported by Paris and Rogers (1996). Using site-directed mutagenesis in the overlapping region of the two contiguous EST clones R30384 and R90202, we constructed the full-length *AtELP*. An extensive RNA and genomic DNA analysis (by PCR and Southern analysis using probes and primers from various regions of the two cDNAs) all indicated that the two contiguous cDNAs are derived from the same gene.

The full-length nucleotide sequence of BP-80 from pea and homologous sequences from maize and Arabidopsis were deposited by J. Rogers in the GenBank database in December, 1996, and this is acknowledged in our paper (Ahmed et al., 1997). These sequences are also reported in a paper by Paris et al. (1997). The BP-80 from pea and AtELP sequences show 70% identity at the amino acid level when several gaps are introduced. The function of AtELP is not known at the moment.

Abbreviation: EST, expressed sequence tag.

LITERATURE CITED

Ahmed SU, Bar-Peled M, Raikhel N (1997) Cloning and subcellular location of Arabidopsis receptor-like protein that shares common features with protein-sorting receptors of eukaryotic cells. Plant Physiol **114:** 325–336

Kirsch T, Paris N, Butler JM, Beevers L, Rogers JC (1994) Purification and initial characterization of a potential vacuolar targeting receptor. Proc Natl Acad Sci USA **91:** 3403–3407

Kirsch T, Saalbach G, Raikhel NV, Beevers L (1996) Inter-

action of a potential vacuolar targeting receptor with amino- and carboxyl-terminal targeting determinants. Plant Physiol **111:** 469–474

Newman T, de Bruijn FJ, Green P, Keegstra K, Kende H, Mcintosh L, Ohlrogge J, Raikhel NV, Somerville S, Thomashow M, and others (1994) Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones. Plant Physiol **106:** 1241–1255

Paris N, Rogers JC (1996) The role of receptors in targeting soluble proteins from the secretory pathway to the vacuole. Plant Physiol Biochem **34:** 223–227

Paris N, Rogers SW, Jiang L, Kirsch T, Beevers L, Phillips TE, Rogers JC (1997) Molecular cloning and further characterization of a probable plant vacuolar-sorting receptor. Plant Physiol **115:** 29–39